REMARKS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

The specification has been amended to make reference to SEQ ID NO:34 in connection with Figure 8B and to correct typographical errors (including the error noted by the Examiner). No new matter has been added. The specification has been also amended to include the Sequence Listing submitted herewith on separate sheets. Entry of the Sequence Listing does not raise the issue of new matter as the sequence information contained therein is presented in the application as originally filed. The computer readable copy of the Sequence Listing submitted herewith is believed to be the same as the attached paper copy of that Listing.

The newly added claims find support throughout the application, including at page 13, last 2 paragraphs.

On page 2 of the Action, the Examiner objects to the drawings. Submitted herewith on separate sheets are copies of Figures 6A, 8A and 8B bearing hand-amendments (in red) corresponding sequence identifiers with the sequences depicted. Approval of the indicated changes is requested.

On page 3 of the Action, the Examiner indicates that the status of the parent case should be recited on page 1.

The parent case, however, is a provisional application. Accordingly, no revision is believed necessary. Clarification is requested.

Claim 5 stands rejected under 35 USC 112, first paragraph, as allegedly being non-enabled. Withdrawal of the rejection is submitted to be in order for the reasons that follow.

In rejecting the claim as non-enabled, the Examiner states that extrapolation from "the teaching of the specification to the enablement of the claims" is not possible because:

"(1) the enablement of the claimed invention is based solely on *in vitro* Dunning Model cultured cell line data,

(2) the specification provides insufficient guidance on the ability of the claimed assay to determine the likelihood (that is predict) metastasis of a prostate tumor in a human".

At the outset, Applicants direct attention to the fact that the subject specification clearly indicates that the loss of the FGF-R2 IIIb isoform and expression of the FGF-R2 IIIc isoform provides a marker for progression of human prostate cancer from an androgen sensitive to an androgen insensitive tumor. In human prostate tumors, androgen-insensitivity is correlated with increased

metastatic potential. Claims 5 and 7 are drawn to methods of determining the likelihood of metastasis of a human prostate tumor and new claim 6 and 8 are drawn to methods of assessing androgen-sensitivity of such a tumor.

As noted above, the Examiner contends that the enablement provided is based solely on *in vitro* Dunning Model cultured cells. Respectfully, such is not the case.

Example 1 describes the identification of a *cis*-acting element in rat FGF-R2 that is required for proper splicing regulation of the mutually exclusive IIIb and IIIc exons. This 57-nucleotide element ('ISAR') was shown to cause both activation of the upstream IIIb exon and repression of the downstream IIIc exon, effects exclusive to DT3 cells (a well differentiated androgen-dependent rat prostate cancer line). Within this sequence, 18 nucleotides were identified that appear to be the most critical for regulation of splicing. This sequence was shown to be highly similar to a similarly situated sequence in the human FGF-R2 gene which has been shown to mediate IIIb activation.

In addition to Example 1, the subject specification provides an extensive description as to how to practice the claimed methods. In this regard, the Examiner's attention is directed to the disclosure beginning at page 14.

(Indeed the Examiner acknowledges that the specification teaches techniques for detection of the FGF-R2 IIIc transcript (as well as antibody techniques for detection of proteins translated from the transcript).)

Furthermore, incorporated by reference at page 18 is Carstens et al, Oncogene 15:3059 (1997). As pointed out in the Amendment filed August 12, 2002, the studies described in that article relate to 3 well characterized human prostate cell lines and 3 metastatic human prostate tumors, maintained as xenografts. It was shown that in both the 2 androgen independent cell lines and the androgen independent xenograft, androgen insensitivity was correlated with loss of the FGF-R2 (IIIb) isoform.

In view of the above, it will be apparent that the experimental data provided is, in fact, not based only on Dunning Model cell lines. Further, the sufficiency of the teachings provided in the Detailed Description of the subject application is evidenced by the *in vitro* and *in vivo* data provided in the Carstens et al article (which too forms part of the disclosure in view of the incorporation by reference).

The Examiner's comments regarding the Carstens et al article are noted. However, those comments overlook the

fact that the authors state, in this peer-reviewed article, that:

> In sum, these results in xenografts, like those in cultured prostatic cell lines, show a correlation between loss of IIIb isoform of FGF-R2 and androgen insensitivity in human prostate tumors.

(emphasis added - see page 3063, left column)

The finding that in PC-3 cells the FGF-R2 gene appears either to be not expressed or somehow has been lost in no way negates this fundamental finding.

Finally, the Examiner is reminded that androgen-insensitivity is correlated with metastatic potential of human prostate tumors (in this regard, see the introduction of the Carstens article discussed above and the introduction of the Yan et al article (of record)). In view of this accepted correlation, no basis is seen for the Examiner's apparent suggestion that the Carstens et al data (*in vivo* and *in vitro*) are not relevant to metastatic potential of human prostate tumors.

In view of the above, reconsideration is requested.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached pages are captioned "**Version With Markings To Show Changes Made**."

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraph beginning at page 12, line 11:

Figures 8A and 8B. Intron sequences important for regulation of rat and human FGF-R2 splicing display are highly similar. (Fig. 8A) Rat intron sequences corresponding to previously reported 21 nucleotide human sequence, IAS 2, which also mediates IIIb activation contain only one nucleotide difference (SEQ ID NOs:45 and 46, respectively). (Fig. 8B) The 57 nucleotide rat ISAR sequence (SEQ ID NO:34) is highly similar to human sequences (SEQ ID NO:47) in this same region, including the 18 nt shown to be most important for regulation (boxed sequences).

The paragraph beginning at page 12, line 21:

FIG. 9. Depiction of a model which can account for results and the high fidelity of FGF-R2 splicing. AT3 cells use a default splicing pathway and choose the IIIc exon because of its stronger [poypyrimidine] polypyrimidine tract (ppt), they splice IIIb inefficiently due to its

weaker polypyrimidine tract. DT3 cells require regulatory factor(s) which can activate (+) the weaker IIIb exon and at the same time repress use of the IIIc exon. The ISAR element (indicated by the hatched box) is shown binding a factor or complex of factors (large shaded oval) which mediates both of these effects. The previously demonstrated contributions of other cis-elements and associated factors (smaller shaded ovals) on IIIb activation are also shown, as well as the suggestion of possible cooperative interaction between proteins bound at several locations within the intron. Abbreviations are defined in the description to Fig. 3.

The paragraph beginning at page 13, line 15:

Progression of human prostate cancer from an androgen sensitive to an androgen insensitive tumor is accompanied by a change in [alterative] alternative splicing of FGF-R2. This change results in a loss of the FGF-R2 IIIb isoform and predominant expression of the FGF-R2 IIIc isoform. This event provides an important biological marker for progression of prostate cancer.

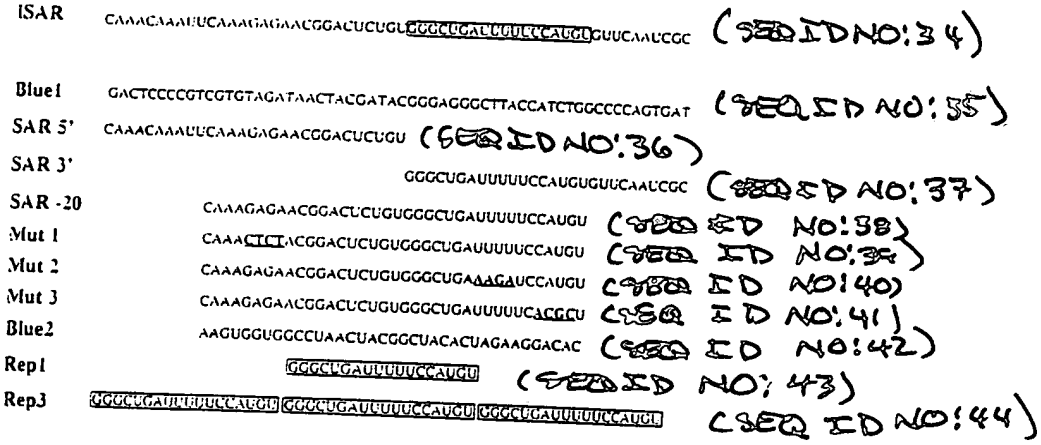
The paragraph beginning at page 31, line 19:

Sequences between the Nde I and Nsi I sites mediate regulation in DT3 cells by activating use of the upstream IIIb exon as well as by repressing use of the downstream IIIc exon. The results obtained with deletions in intron 2 suggested that the requirements for exon IIIc inclusion in AT3 cells are less stringent than those for IIIb inclusion in DT3 cells. In fact, with the sets of minigenes no intronic sequences outside of the conserved splice junctions or [poypyrimidine] polypyrimidine tract were observed which impeded splicing of IIIc in AT3 cells. This is not surprising given the stronger polypyrimidine tract associated with the IIIc exon when compared to that of IIIb. Thus, while the possibility exists that there are other untested intron sequences or exon IIIc sequences which interact with AT3 cell-specific factors to mediate IIIc inclusion in these cells, it is expected that IIIc exon inclusion is a default splicing pathway which may only require the cooperation of factors involved in the constitutive splicing process. Thus, regulation may be achieved by proteins in DT3 cells which are able to switch the splicing pattern from exon IIIb to IIIc. Consistent with this view are the observations that several of the

deletions caused not only skipping of both exons, but also a switch towards some IIIc inclusion. Therefore, if FGF-R2 mutually exclusive alternative splicing is predominantly regulated only in DT3 cells, the sequences which are involved in this regulation could be acting by activating IIIb splicing, repressing IIIc splicing, or by performing both of these functions. To investigate these alternatives, a series of minigenes were constructed in which either IIIb or IIIc (but not both) was inserted into pI-11 and the previous deletions were used in such a manner that IIIb was inserted either with (pI-11-IIIb-plus) or without (pI-11-IIIb-minus) the Nde I to Nsi I sequences located downstream, and IIIc was inserted with (pI-11-IIIc-plus) or without (pI-11-IIIc-minus) these same sequences upstream (Fig. 5A). Because these minigenes only offered a choice of including an internal exon or skipping, the use of the internal IIIb or IIIc exon was quantified vs. that of the skipped product. As shown in Fig. 5B, when these minigenes were transfected into AT3 cells, the IIIc exon was included highly efficiently, and this inclusion was not affected by the presence of the Nde I to Nsi I sequences located upstream. In addition, AT3 cells did not include exon IIIb efficiently and this effect was essentially unchanged whether or not these sequences were located

downstream. In DT3 cells, on the other hand, IIIb inclusion was seen to occur with fairly high efficiency, but this inclusion was largely dependent on the presence of the Nde I to Nsi I sequence located downstream; when this sequence was deleted IIIb inclusion was dramatically reduced from 68% to only 13%. In addition, it was noted that when the Nde I to Nsi I sequences were present upstream of IIIc, DT3 cells included IIIc rarely, but when these sequences were deleted, the proportion of IIIc included approximately tripled, from 11% to 35%. This data was consistent with a model in which regulation of FGF-R2 alternative splicing in DT3 cells is achieved by the interaction of a cell-specific factor or complex of factors that interact with intronic sequences in intron 2 and coordinated activation of exon IIIb splicing and repression of the stronger IIIc exon. The fact that the sequences between Nde I and Nsi I were necessary for both of these effects to occur resulted in this sequence being designated ISAR (Intronic Splicing Activator and Repressor) and indicated that this element is required for the formation of a regulatory complex which acts in DT3 cells to force the use of IIIb instead of IIIc. (The sequence between exons IIIb and IIIc is set forth in Fig. 10 (rat) and Fig. 11 (human)).

6 A



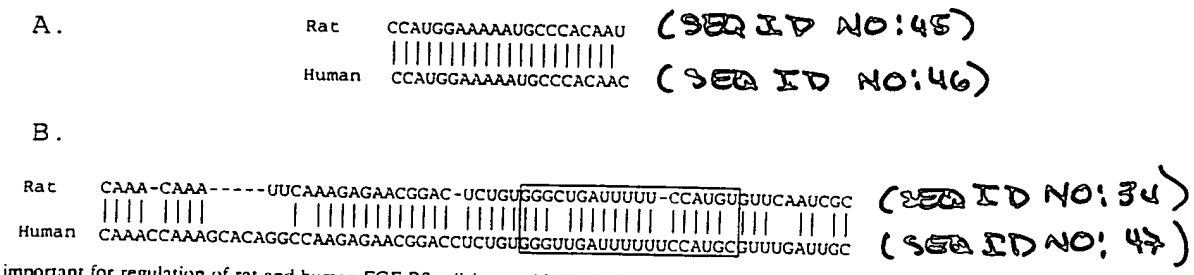


FIG. 8. Intron sequences important for regulation of rat and human FGF-R2 splicing are highly similar. (A) Rat intron sequences corresponding to a previously reported 21-nucleotide human sequence, IAS2 (see Results), which also mediates IIIb activation, contain only 1 nucleotide difference. (B) The 57-nucleotide rat ISAR sequence is highly similar to human sequences in this same region, including the 18 nucleotides shown to be most important for regulation (boxed sequences).